47. (amended) The kit of claim 46 wherein the anti-Apo A-I and anti-Apo A-II monoclonal or recombinant antibody [molecule specifically immunoreactive with a single specific lipoprotein or apolipoprotein is] molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

Remarks

Rejections under 35 U.S.C. 112

Claim 39 was rejected under 35 U.S.C. 112 as containing subject matter which was not described in the specification. This rejection is respectfully traversed. The support for each portion of claim 39 is noted below:

39. A method for determining the relative ratio of LDL to HDL in a biological sample comprising

determining the amount of LDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein (see example 7, pages 60-62 for anti-LDL);

determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of

high density lipoprotein (see example 8, pages 63-63); and

determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein (see example 9, pages 65-66).

Claims 1-12, 40, 41, 43, 45, and 47 were rejected under 35 U.S.C. 112 as indefinite. Claims 1-11 have been amended to refer to apolipoproteins or specific lipoproteins, LDL, HDL, or VLDL. Claim 2 has been amended to narrow the scope from claim 1 which includes VLDL to recite only LDL or HDL. Claims 3-5 have been amended to clarify antecedent basis of the antibodies. Claims 6-8 have been amended to refer to a further step wherein the amount of bound lipid is determined by staining with Sudan B. Claim 9 has been amended to recite that the antibody coupled to a protein stain and to correct antecedent basis as suggested by the examiner.

Claim 12 has been amended to recite that the method involves binding to two monoclonal antibodies to different apolipoproteins, then separation of the two bound apolipoproteins by binding of one of the apolipoproteins to a third, immobilized antibody which is immunoreactive with a different epitope on the apolipoprotein than that bound by one of the monoclonal antibodies in solution. Claim 13 has been amended to more clearly recite the referenced antibodies.

Claims 40 and 41 have been amended to clarify the reaction components and reaction products. The word "predominantly" has been deleted but it is believed the word is not indefinite as used in the claims, and those skilled in the art will understand the meaning of the claims from the specfication. Claims 43, 45, and 47, and the claims they depend from, have been

amended to correct antecedent basis.

Rejections under 35 U.S.C. 102 and 103

Claims 46 and 47 were rejected under 35 U.S.C. 102(b) as disclosed by U.S. Patent No. 4,677,057 to Curtiss, et al. Claims 1, 2, 3, 10 and 11 were rejected under 35 U.S.C. 103 as obvious over U.S. Patent No. 5,126,276 to Fish, et al., in combination with EP 0262854 to Scripps and Forster, et al., Biochem. Soc. Trans. 18(6):1180(1990) and Zhow, et al., Hubi Yixueyuan Xueabo. II(4), 298-302 (1998). Claim 10 was rejected under 103 as obvious over Fish in combination with Scripps, Forster, and Zhow, and further in view of Koren, et al., Atherosclerosis 95, 157-170 (1992). Claims 1, 2, 3, and 6 were rejected under 103 as obvious over Fish, Scripps, Forster, and Zhow and further in view of EP 0407 035 to Luca. Claims 7 and 8 were rejected under 103 as obvious over Fish, Scripps, Forster, and Zhow, and further in combination with Mills, et al. Laboratory Techniques in biochemistry and molecular biology, vol. 14, pages 472-478 (1984). Claims 42-45 were rejected under 35 U.S.C. 103 as obvious over Koren, et al., (1992) (presumably in combination with Curtiss). These rejections are respectfully traversed.

Claims 46 and 47 recite:

46. A kit for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

monoclonal or recombinant Apo-A-I antibody specifically immunoreactive with Apo A-I lipoproteins in human plasma; and

monoclonal or recombinant Apo A-II antibody specifically immunoreactive with Apo A-II,

wherein the anti-Apo A-I or anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

47. The kit of claim 46 wherein the anti-Apo A-I and anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

The claims now require that one or both of the antibodies bind to an epitope that is uninfluenced by lipid content. Curtiss clearly fails to disclose such antibodies. As described at col. 13, lines 5-66, demonstrates that binding of each of the four antibodies (three anti-Apo I, and one anti-Apo II) were affected by either conformation and/or lipid content of the lipoprotein. See also col. 14, lines 58-68, further demonstrates that Curtis did NOT expect to produce antibodies which bound in a conformation, lipid-independent manner.

Claims 1, 2, 3, 10 and 11 define a method for determining the relative ratio of LDL to HDL or at least two different apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second monoclonal antibody molecules immunoreactive with LDL, HDL or VLDL or at least two different apolipoproteins, wherein the first and second monoclonal antibodies bind to either LDL, HDL or VLDL or to different apolipoproteins in a conformation and lipid content independent manner;

allowing the monoclonal antibody molecules time to bind to the LDL, HDL or VLDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized monoclonal antibody molecules;

determining the amount of LDL, HDL or VLDL lipoprotein or at least two different apolipoproteins bound by the immobilized monoclonal antibody molecules, and

comparing the amount bound which is specific for LDL, HDL or VLDL or each apolipoprotein in order to calculate the relative amounts of LDL, HDL or VLDL or apolipoproteins.

Fish shows substrates suitable for use of immobilized antibodies which can be dipped into a sample solution to bind to antigen. There is no specific disclosure using antibodies to LDL, HDL or VLDL on the same substrate to provide a comparative ratio.

Scripps describes assays in which the binding of Apo-B100 relative to the amount of ApoAI is compared. Scripps notes that Apo B-100 is found in LDL and that Apo AI is found in HDL (page 3, lines 1-6 and lines 28-30). At page 4, lines 48-51, the authors also note that Apo

AI is found in **LDL**. Therefore, there is no disclosure of antibodies which are reactive only with LDL OR HDL OR VLDL. There is also no disclosure of antibodies which are reactive with at least two different apolipoproteins where the antibodies to the apolipoproteins bind with an affinity which is conformation and lipid independent. This problem is described by Fish at page 7, lines 9-14. As described at page 13, lines 10-14, the antibodies to Apo B100 reacted with LDL, VLDL and IDL. As described at page 17, lines 10-20, binding of lipid (as in chylomicrons) affects binding to Apo B100. The data at page 18, lines 12-18, indicates that binding of the anti-Apo AI antibodies is affected by lipid, therefore they are not lipid and conformation independent. In contrast, plasma or serum samples can be used undiluted with the conformation and lipid independent antibodies described by applicant. Therefore the antibodies of claim 1 and claims dependent thereon are not disclosed by Fish. The assay utilized by Fish (see page 8, lines 1-26) is very complex as a result of the problems that arise as a result. As the examiner is aware from the prosecution of applicants' related applications, it was applicants' development of a technique to produce lipid and conformation independent antibodies that was critical to development of the claimed assays and kits for use therein.

Forster describes the desirability of a two antibody assay to obtain the ratio of HDL to LDL but provides no details as to how such an assay could be done or what antibodies are used.

Zhow merely demonstrates that ratios of Apo AI to Apo B may be useful in the diagnosis of heart disease.

Assuming one were motivated to determine the ration of Apo AI to Apo B, one would

still not have the claimed assay. The prior art antibodies fail to completely distinguish between VLDL, LDL and HDL, or the individual apolipoproteins under conditions of varying lipid concentration or conformation. The claims have been amended to clarify and more clearly define these difference.

As noted on page 29, lines 3-13, Koren (1992) describes antibodies to Apo CIII and Apo E. These are not described as conformation and lipid independent antibodies, and do not completely distinguish between lipoproteins (note the use of the modifying term "predominantly"). Even in combination with the other prior art, one would not achieve the claimed assays.

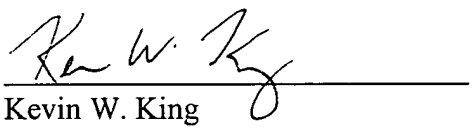
Lucas does not make up for this deficiency. Lucas also recognizes the desirability of determining the relative amounts of HDL and LDL, as well as apolipoproteins. The assay requires immobilization of the intact lipoproteins so that the other sample components can be removed. Lucas says (col. 10, lines 9-12) that monoclonals are better for recognition of specific epitopes. The need to remove other sample components clearly indicates that the other sample components would have an effect on the assay as Lucas envisions it, so the antibodies cannot be lipid and conformation independent. Moreover, based on the disclosure at col. 16, lines 51-60, the immunogens are not treated to delipidate and solubilize and reduce the molecules so that one could obtain antibodies that are reactive with lipid and conformation independent epitopes.

Mills does teach staining of lipids. However, as discussed above, this would still not lead those skilled in the art to the claimed methods and kits for use therein.

Curtis and Koren are both discussed above. Neither teaches antibodies which are lipid and conformation independent, nor methods for making such antibodies. Neither teaches assays which are conformation and lipid independent. Therefore the claims to the kits for use in such an assay are not obvious from the combination thereof.

Allowance of claims 1-13 and claims 39-47 is earnestly solicited. All claims as pending upon entry of this amendment are attached in an Appendix to facilitate review by the Examiner.

Respectfully submitted,



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Amendment

CERTIFICATE OF MAILING (37 CFR 1.8a)

I hereby certify that this paper, along with any paper referred to as being attached or enclosed, is being deposited with sufficient first class postage in an envelope addressed to the Assistant Commissioner of Patents, Washington, D.C. 20231 on the date shown below.

Date: November 27, 2000

Jean Hicks

APPENDIX: Claims as Pending Upon Entry of the Amendment

1. (twice amended) The method for determining the relative ratio of LDL to HDL or at least two different apolipoproteins in a biological sample comprising:

immersing into the sample a solid phase material having separately immobilized thereon at least first and second monoclonal antibody molecules immunoreactive with [a specific lipoprotein indicative of] LDL [or], HDL or VLDL or at least two different apolipoproteins, wherein the first and second monoclonal antibodies bind to either LDL, HDL or VLDL or to different apolipoproteins in a conformation and lipid content independent manner;

allowing the monoclonal antibody molecules time to bind to the [lipoprotein in the] LDL [and], HDL or VLDL or apolipoproteins in the sample;

removing the solid phase material containing the immobilized monoclonal antibody molecules;

determining the amount of LDL [and], HDL or VLDL lipoprotein or at least two different apolipoproteins bound by the immobilized monoclonal antibody molecules, and

comparing the amount bound which is specific for LDL [or], HDL or VLDL or each apolipoprotein in order to calculate the relative amounts of LDL [and], HDL or VLDL or apolipoproteins.

2. (amended) The method of claim 1 wherein the antibody molecules immobilized on the solid phase material are immunoreactive with [a] lipoproteins selected from the group consisting of HDL[,] and LDL[, VLDL, and combinations thereof].

3. (twice amended) The method of claim 2 wherein the [antibody is] antibodies to the HDL or LDL are selected from the group consisting of recombinant antibodies and antibody fragments.

4. (amended) The method of claim 3, wherein the [antibody is] antibodies comprise the anti-LDL monoclonal antibody produced by the hybridoma cell line HB_3cB_3 ATCC designation number HB 11612.

5. (amended) The method of claim 3, wherein the [antibody is a] antibodies comprise recombinant anti-LDL $RcB_3M_1D_4$ ATCC designation number 69602.

6. (twice amended) The method of claim 1 [wherein] further comprising determining the amount of lipoprotein or lipid associating with apolipoprotein [is determined] by staining of the material bound to the immobilized antibody using a lipid stain.

7. The method of claim 6 wherein the lipid stain is selected from the group consisting of Sudan Red 7B, Oil Red O, and Sudan Black B.

8. The method of claim 6 wherein the lipoprotein lipid is stained prior to immersing the immobilized antibodies.

9. (twice amended) The method of claim 6 further comprising measuring the amount of apolipoprotein or protein associated with lipid in the sample, further comprising the

step of providing [a third antibody immunoreactive with apolipoprotein wherein the third] antibody [is] immunoreactive with the apolipoproteins coupled to a protein stain [and used to stain lipoprotein] , and staining the apolipoprotein or protein associated with lipid in the sample, [prior to immersing into the sample the immobilized first antibodies which then bind to the stained second antibody-bound apolipoprotein].

10. The method of claim 1, wherein the apolipoprotein is selected from the group consisting of Apo A-I, Apo A-II, Apo B, Apo C-III, and Apo E.

11. The method of claim 1, wherein the biological sample is selected from the group consisting of blood, plasma, and serum.

12. (twice amended) A method of determining the relative concentration of at least two different apolipoproteins in a biological sample comprising:

mixing in solution a first and second monoclonal antibody molecules each immunoreactive with a specific apolipoprotein into the sample, wherein at least one of the first and second monoclonal antibodies bind to different apolipoproteins in a conformation and lipid content independent manner;

allowing the monoclonal antibody molecules to bind to the apolipoprotein in the sample,

immersing into the mixture third immobilized monoclonal antibody molecules immunoreactive with a second, distinct epitope of [an apolipoprotein] one of the apolipoproteins,

allowing the third immobilized monoclonal antibody molecules to bind to the apolipoprotein,

detecting the presence of the apolipoprotein bound by both [both] one of the first and second monoclonal antibodies and the third immobilized monoclonal antibodies, and

determining the amount of [each] apolipoprotein bound by both one of the first and second monoclonal antibodies and the third immobilized monoclonal antibodies.

13. (amended) The method of claim 12 wherein the apolipoprotein bound by one of the monoclonal antibodies in solution is apolipoprotein Apo B-100.

39. (amended) A method for determining the relative ratio of LDL to HDL in a biological sample comprising

(a) determining the amount of LDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with low density lipoprotein and not cross-reactive with high density lipoprotein and determining the amount of low density lipoprotein;

(b) determining the amount of HDL in the sample by

adding to the sample monoclonal antibody molecules immunoreactive with high density lipoprotein and not cross-reactive with low density lipoprotein and determining the amount of high density lipoprotein; and

(c) determining the ratio of the amount of low density lipoprotein with the amount of high density lipoprotein, wherein at least one of the monoclonal antibodies to LDL and HDL bind in a conformation and lipid content independent manner.

40. (amended) A method for determining the relative ratio of VLDL to HDL in a

biological sample comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo C-III present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the anti-ApoC-III antibody reactive with Apo C-III with the biological sample to form complexes between the anti-ApoC-III antibody and the Apo C-III containing lipoprotein particles,

contacting the Pan B antibody with the biological sample containing the anti-ApoC-III antibody bound to the Apo C-III containing lipoprotein particles,

separating the complexed Pan B-anti-ApoC-III antibody-lipoprotein particles from the biological sample, [and

determining] which is the amount of Apo C-III [associated with Apo B, which is the amount of Apo C-III] present in VLDL in the anti-Apo C-III anti-Apo B complexed material in the sample; and

(b) determining the amount of HDL in the sample by

determining the amount of Apo C-III present in the HDL in the sample by

providing Apo A-I monoclonal antibody specifically immunoreactive [specifically] with Apo A-I,

providing monoclonal antibody specifically immunoreactive with Apo C-III,

contacting the antibody reactive with Apo C-III with the biological sample to form complexes between the anti-Apo C-III antibody and the Apo C-III containing lipoprotein particles,

contacting the anti-Apo A-I antibody with the biological sample to form complexes with the anti-Apo C-III antibody-Apo C-III containing lipoprotein particles,

separating the complexed antibody-lipoprotein particles from the biological sample,

determining the amount of Apo C-III [associated with Apo A-I, which is the amount of Apo C-III] present in HDL in the anti-Apo C-III-anti-Apo A-I complexed material in the sample, and

determining the ratio of Apo C-III present in VLDL in the sample and Apo C-III present in HDL in the sample, which is the ratio of VLDL to HDL,

wherein the VLDL and HDL are measured in the same sample using immobilized anti-Apo A-I and anti-Apo B or anti-Apo C-III antibodies or measured by immunoprecipitation with the anti-Apo A-I and anti-ApoB antibodies or anti-Apo C-III antibodies in separate samples,

wherein at least one of the monoclonal antibodies bind to Apo AI, Apo B, or Apo CIII in a conformation and lipid content independent manner.

41. (amended) A method for determining the relative ratio of VLDL to HDL comprising

(a) determining the amount of VLDL in the sample by

determining the amount of Apo E present in the VLDL in the sample by

providing Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

providing monoclonal antibody which specifically binds to Apo E associated [predominantly] with VLDL,

contacting the antibodies reactive with Apo E associated with VLDL with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample containing the complexes between the anti-ApoE antibodies and ApoE containing particles to form complexes of anti-ApoB-anti-ApoE-ApoE containing particles, and

determining the amount of Apo E [associated with Apo B] in the complexes of anti-ApoB-anti-ApoE-ApoE containing particles, which is the Apo E present [predominantly] in VLDL in the sample;

(b) removing the [complexed anti-Apo E:Pan B:Apo E] complexes of anti-ApoB-anti-ApoE-ApoE containing particles, either by binding [by immobilization] of the anti-Apo E antibodies to an immobilized surface or centrifugation of [complexed particles] sample to remove the complexes of anti-ApoB-anti-ApoE-ApoE containing particles;
and

(c) determining the amount of HDL in the sample by

determining the amount of Apo E present in the HDL in the sample by

providing Apo A-I monoclonal antibody immunoreactive specifically with Apo A-I,

providing monoclonal antibody which binds to Apo E [predominantly] associated with HDL,

contacting the antibodies reactive with Apo E [to] with the biological sample to form complexes between the anti-ApoE antibodies and Apo E containing particles,

contacting Pan B antibody with the biological sample for form complexes of the anti-ApoE antibodies-ApoE containing particles-anti-ApoB,

determining the amount of Apo E [associated with Apo A-I, which is the amount of Apo E] present in HDL in the complexes of the anti-ApoE antibodies-ApoE containing particles-anti-ApoB in the sample, and

determining the ratio of Apo E present in VLDL in the sample and Apo E present in HDL in the sample which is the ratio of VLDL to HDL,

wherein at least one of the monoclonal antibodies bind to Apo B, Apo AI, or Apo E in a conformation and lipid content independent manner.

42. (amended) A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal or recombinant antibody specifically immunoreactive with Apo C-III, and

monoclonal or recombinant Apo A-I antibody specifically immunoreactive with Apo A-I,

wherein at least one of the monoclonal or recombinant antibodies bind to Apo B, Apo AI,

or Apo CIII in a conformation and lipid content independent manner.

43. (amended) The kit of claim 42 wherein the anti-Apo C-III or anti-A-1 monoclonal or recombinant antibody molecules [specifically immunoreactive with a single specific lipoprotein or apolipoprotein] are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

44. (amended) A kit for determining the relative ratio of VLDL to HDL comprising

Pan B antibody which is characterized by an equal binding and high affinity for all Apo B-containing lipoproteins in human plasma,

monoclonal antibody which predominantly binds to Apo E associated with VLDL ,

monoclonal Apo A-I antibody specifically immunoreactive with Apo A-I, and

monoclonal antibody which predominantly binds to Apo E in HDL.

45. (amended) The kit of claim 44 wherein the anti-Apo E or anti-Apo A-I monoclonal or recombinant antibody molecules [specifically immunoreactive with a single specific lipoprotein or apolipoprotein] are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

46. (amended) A kit for determining the relative ratio of LPA-I and LPA-II lipoprotein particles comprising

monoclonal or recombinant Apo-A-I antibody specifically immunoreactive with Apo A-I lipoproteins in human plasma; and

monoclonal or recombinant Apo A-II antibody specifically immunoreactive with Apo A-II,

wherein the anti-Apo A-I or anti-Apo A-II monoclonal or recombinant antibody molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.

47. (amended) The kit of claim 46 wherein the anti-Apo A-I and anti-Apo A-II monoclonal or recombinant antibody [molecule specifically immunoreactive with a single specific lipoprotein or apolipoprotein is] molecules are selected from the group consisting of monoclonal antibodies, recombinant antibodies, and monoclonal antibody fragments that specifically bind to a stable, conformation independent epitope which is uninfluenced by the lipid content of the lipoprotein, apolipoprotein, or lipid associated with a specific lipoprotein.